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ACTIVATED MACROPHAGE-CONDITIONED MEDIA INDUCES FIBROGENESIS IN A GASTROINTESTINAL SCAR-IN-A-JAR MODEL THAT IS QUANTIFIABLE WITH SEROLOGICAL BIOMARKERS OF COLLAGEN FORMATION

M. Pehrsson[1], F. Gillesberg[1,2], Y. Lou[3], T. Tsapanou-Katranara[1,4], E. Mi[3], A.C. Bay-Jensen[1], S. Holm Nielsen[1], S. Khan[5], M.S Alexdóttir[1], H.S. Hector[1], M. Karsdal[1], J.H. Mortensen[1]
[1]Nordic Bioscience, Denmark; [2]Aarhus University, Denmark; [3]Bristol-Myers Squibb, United States of America; [4]University of Southern Denmark, Denmark; [5]University of Copenhagen, Denmark

Background

- Intestinal fibrosis affects most inflammatory bowel disease (IBD) patients, resulting in severe clinical complications and reduced treatment response.
- Myofibroblasts are the main drivers of intestinal fibrosis and the excessive accumulation of extracellular matrix in IBD patients.
- With no treatments approved for intestinal fibrosis, there is a need for preclinical models to investigate the pathobiology and novel treatments.

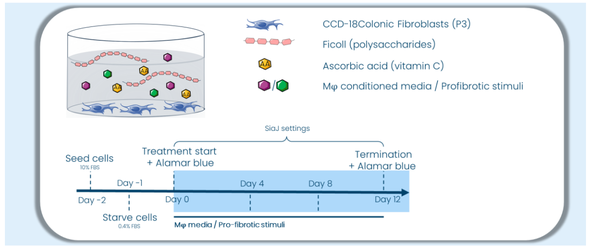
Aims

- We investigated the profibrotic effects of activated macrophages (Mφ) in a scar-in-a-jar model of colonic fibrogenesis using validated protein fingerprint assay (PFA) biomarkers of collagen formation.

Methods

- Mφs isolated from healthy donor buffy coats were polarized to TNF-a producing M1-like Mφs using 25 ng/mL IFN-γ and M-CSF over two rounds (days 0-6 and days 7-12).
- Human colonic fibroblasts (CCD18-Co) cells were seeded at 30,000 cells/well supplemented with Ficoll and Ascorbic acid for a pseudo 3D environment supporting collagen production.
- Mφ M1-like conditioned media was added in ratios of 30%, 10% and 3% of total media to the colonic fibroblasts to evaluate the profibrotic effects.
- As controls, the colonic fibroblasts were stimulated with 50 pM TGF-β, 25 ng/mL IFN-γ + M-CSF or unstimulated FBS control.
- The PFA biomarkers, PRO-C1, PRO-C3, PRO-C6, and FBN-C quantifying type I, III, VI, and fibronectin formation were quantified in the supernatants on days 0, 4, 8, and 12.
- The statistical differences in biomarker levels were calculated using the area under the curve (AUC) compared with the unstimulated FBS control applying Brown-Forsythe and Welch or one-way ANOVA tests.

PFA biomarkers:
- PRO-C1 (Type I collagen formation)
- PRO-C3 (Type III collagen formation)
- PRO-C6 (Type VI collagen formation, endotrophin)
- FBN-C (Fibronectin formation)



Results

- Overall, stimulation with Mφ M1-like conditioned media in ratios of 30%, 10%, and 3% of the total media volume induced formation of type III and VI collagen and fibronectin compared to the FBS control. Type I collagen formation was reduced.
- Mφ conditioned media collected from days 7-12 of Mφ activation induced the highest induction of collagen formation.
- Stimulating human colonic fibroblasts with TGF-β1 induced a significant percentage increase of PFA biomarkers quantifying type I, III, and VI collagen and fibronectin formation (Figure 1)
- The addition of IFN-γ and M-CSF used for Mφ activation inhibited type III collagen formation.

Figure 1: Quantification of fibrogenesis following stimulation with Mφ conditioned media from days 0-6

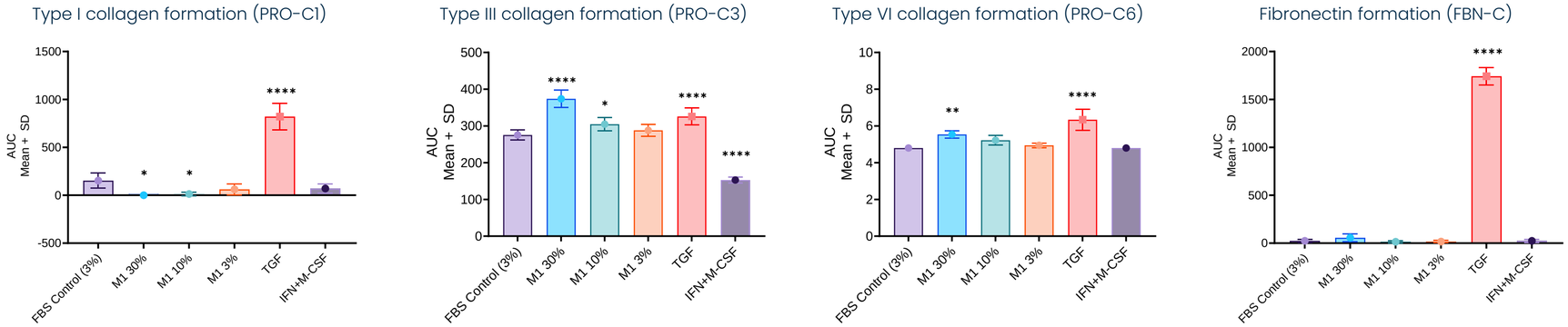
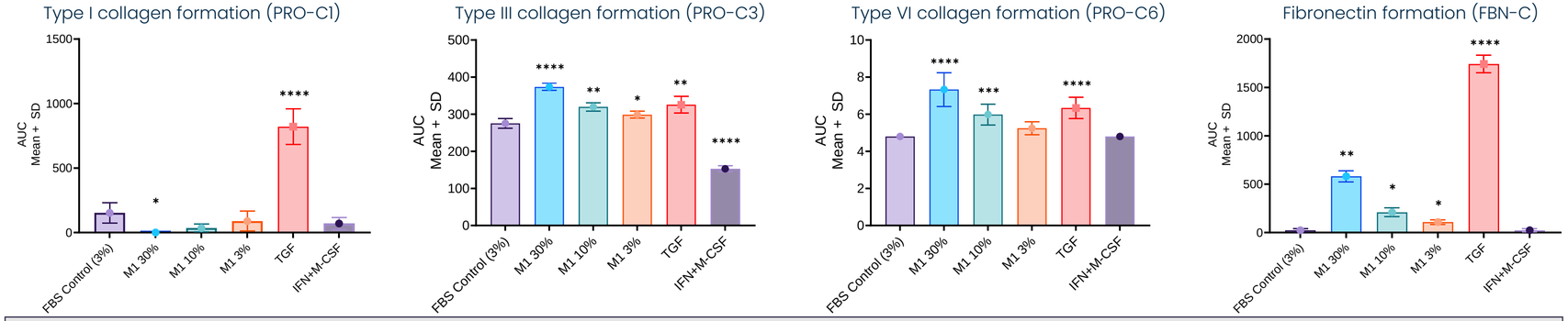


Figure 2: Quantification of fibrogenesis following stimulation with Mφ conditioned media from days 7-12



Major findings and Conclusion

- **Mφ conditioned media induce fibrogenesis in human colonic fibroblasts with increased type III and VI collagen and fibronectin formation.**
- **TGF-β induced formation of type I, III, and VI collagen and fibronectin**
- **The noninvasive PFA biomarkers can be utilized to objectively quantify fibrogenesis in the *in vitro* Scar-in-a-Jar model system to investigate mechanisms of fibrogenesis**

COI: M.P, A.C.B.J, S.H.N, M.S.A, H.S.H, M.A.K, and J.H.M are employed at Nordic Bioscience A/S, Denmark